Correlation between quantitative HER-2 protein expression and risk for brain metastases

Jacek Jassem

Medical University of Gdańsk, Poland

Backround

- Breast cancer carries a high risk of brain relapse
- Brain metastases are associated with poor prognosis, negatively impact quality of life and are resistant to systemic therapies
- Particularly high risk of brain relapse is associated with HER2-positivity (30-50% occurrence in advanced disease)
- Trastuzumab does not cross an intact blood-brain barrier, and is ineffective in preventing and treating brain metastases
- High risk patients might benefit from active surveillance for brain relapse or from preventive strategies
- Reliable clinical or biological factors allowing patient selection for such interventions are lacking

HERmark (VeraTag) Breast Cancer Assay

- **Enables precise quantitative measurements of total HER2 protein expression in formalin-fixed paraffin-embedded tissue specimens**
- **Higher HER2 expression by VeraTag is associated with increased survival in HER2-positive advanced breast cancer patients treated with trastuzumab[1,2]**
- **Association between quantitative HER2 level and the propensity to metastasize to particular sites is unknown**

Lipton et al., Cancer 2010;116:5168-5178
Toi et al. BMC Cancer 2010;10:56.

Study objective

As a part of our research project related to clinical usefulness of HER2 level by VeraTag, we investigated whether quantitatively expressed HER2 protein level is predictive for the risk of brain relapse in HER2-positive advanced breast cancer patients treated with trastuzumab

Study group

- **HER2-positive (IHC 3+ or IHC 2+/FISH-positive) advanced breast cancer patients treated in 9 Polish institutions between December 2000 and July 2010**
- **Brain metastases symptomatic or detected accidentally**
- **Central pathology review**
- **At least one dose of trastuzumab for metastatic disease with or without chemotherapy**
- **In most cases trastuzumab continued until progression**
- **Median follow-up time from the initial diagnosis: 68 months (7-144 months)**
- **Median time from the initiation of trastuzumab-containing therapy: 29 months (1-115 months)**

Methods

- **Dominant site of distant disease classified by the category associated with the worst prognosis, irrespective of the extent of involvement, in the following order of increasing gravity: soft tissue, bones, viscera**
- **HER2-positivity: IHC 3+ or HER2 FISH/CEP17 ratio ≥2.0**
- **FISH assessed centrally in all patients**
- **ER/PR positivity: ≥10% of nuclear staining using IHC**
- **Quantitative HER2 protein levels measured using HERmark assay[1,2], with units of relative fluorescence per mm^2 of invasive tumor (RF/mm^2 tumor)**

[1]Lipton et al., Cancer 2010;116:5168-5178
[2]Larson et al. Patholog Res Int 2010;2010:814176

Statistics

- Time to brain metastasis calculated from initiation of trastuzumab treatment to diagnosis of brain metastasis, or censored due to end of follow up or death
- Overall survival calculated from initiation of trastuzumab treatment to the death (from any cause) or censored due to end of follow up
- Kaplan-Meier method used to estimate the probability of brain metastases over time
- Univariate analysis using log-rank test
- Cox models used for multivariate analysis and to estimate the hazard ratios
- Time to non-brain progression used as a time-dependent variable to examine the effect of other progressions on the risk of brain metastases
- Competing risks of death subjected to additional analyses

Occurrence of brain metastases

- **49 out of 142 patients (35%) developed symptomatic brain relapse**
- **Median time to brain metastasis: 13 months (95% CI: 9-18 months)**
- **In 20 patients brain metastases occurred at the time of first metastatic progression**
- **Cumulative risk of developing brain relapse from initiation of trastuzumab-containing treatment:**

1 year:	**19%**
2 years:	**30%**
3 years:	**46%**

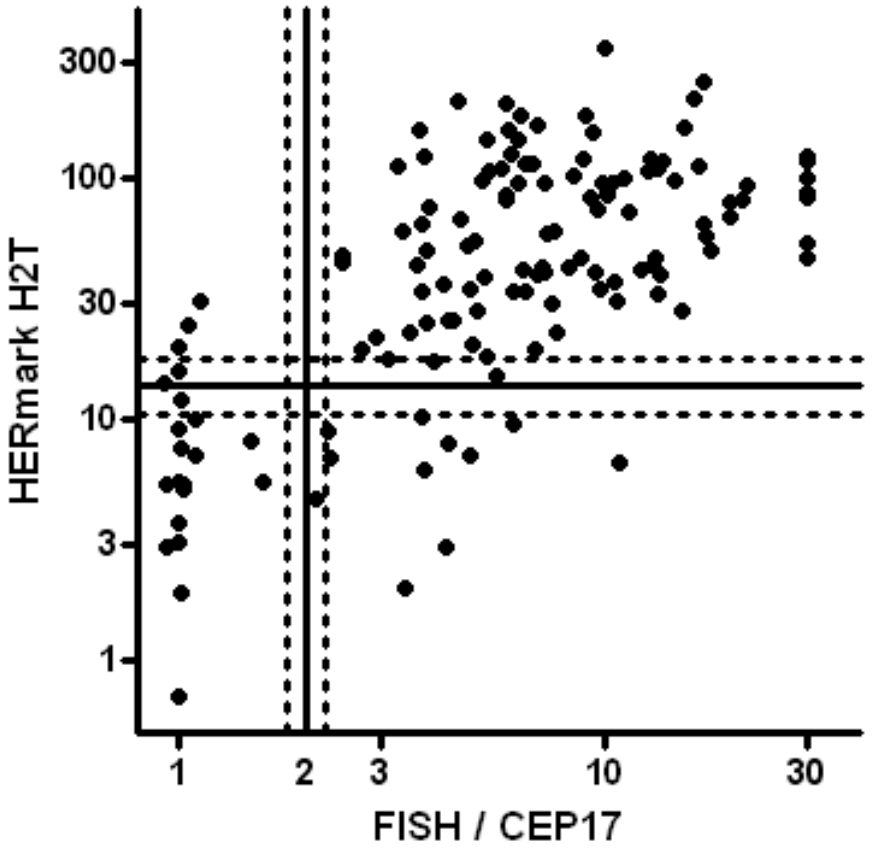
Patient characteristics

Variable	No brain metastases (N=93)	Brain metastases (N=49)	All patients (N=142)
Menopausal status			
Pre-menopausal	44%	47%	45%
Post-menopausal	56%	53%	55%
Dominant metastatic site			
Soft tissue	22%	16%	20%
Bone	22%	8%	17%
Viscera	56%	76%	63%
Unknown	1%	0	1%
ER			
Positive	43%	31%	39%
Negative	57%	69%	61%
Grade			
G3	53%	73%	60%
G1 + G2	47%	27%	40%

Patient characteristics (cont.)

Variable	No brain metastases (N=93)	Brain metastases (N=49)	All patients (N=142)
Pathology type			
Ductal	90%	88%	89%
Lobular	2%	4%	3%
Other	2%	2%	2%
Unknown	5%	6%	6%
HER2 protein (HERmark H2T)			
Positive	67%	96%	77%
Equivocal	8%	0%	5%
Negative	26%	4%	18%
FISH/CEP17			
> 2.0	78%	90%	82%
≤ 2.0	19%	6%	15%
unknown	2%	4%	3%
Age at progression			
Median	54	50	53
Range	25-79	33-72	25-79

Relationship between HER2 protein by HERmark H2T and HER2 FISH



Concordance H2T and FISH/CEP17: 86%; kappa = 0.55, considering negative, equivocal and positive categories for both H2T and the HER-2/CEP17 ratio

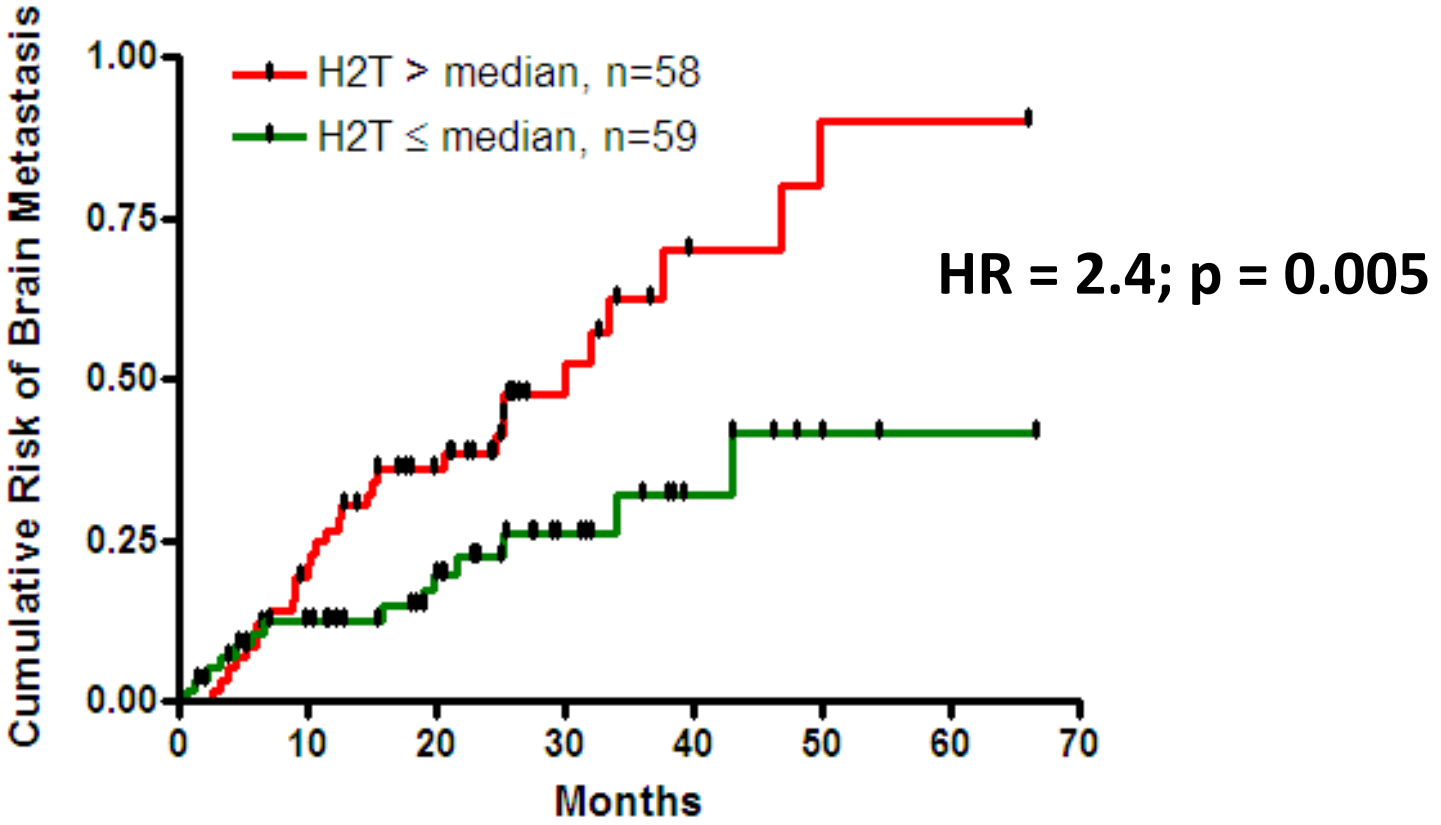
Investigated variables

- Age
- Menopausal status
- Dominant site of metastatic disease
- ER status
- PgR status
- Tumor grade
- Time to non-brain progression
- HER2 status by FISH
  - considered as a categorical variable (using $\geq$2 cutoff)
  - as a continuous variable
- HER2 protein expression (H2T)
  - considered as a categorical variable using specific cutoffs
  - as a continuous variable

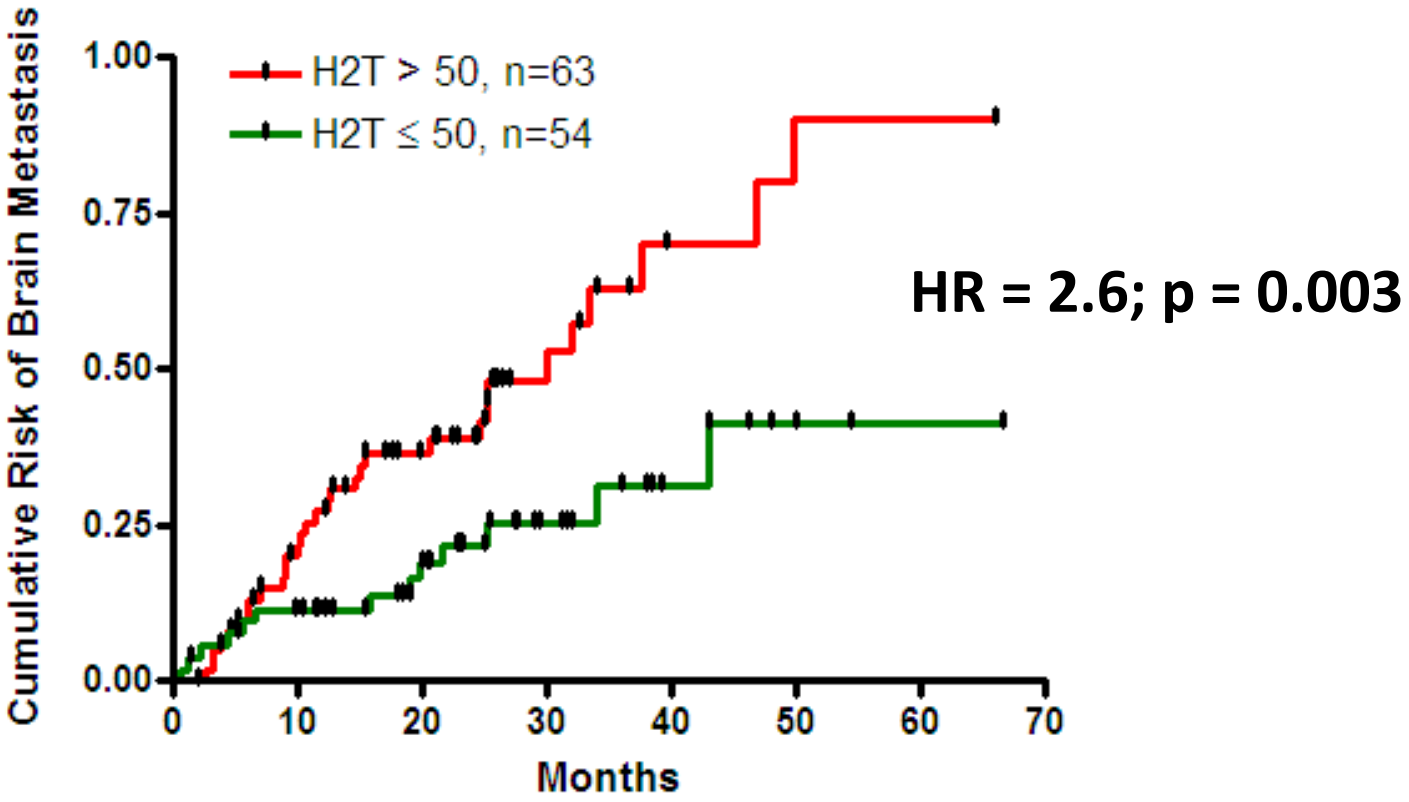
Time to brain metastases: univariate analysis

Variable	Category	HR	P
Age	Continuous	0.99	0.6
Menopausal status	Pre/Postmenopausal	1.03	0.9
Dominant metastases	Viscera/Bone/Soft tissue	-	0.095
ER	Positive/Negative	0.75	0.4
PgR	Positive/Negative	1.1	0.7
Grade	G3/G1 + G2	2.4	0.007
FISH HER2	Positive/Negative	1.9	0.28
Log FISH HER2	Continuous	1.7	0.25
Log HER2 protein (H2T)	Continuous	2.3	0.013
HER2 protein (H2T)	> 50/≤ 50	2.6	0.001
Time to non-brain metastases	Continuous	2.5	0.006

Time to brain metastases according to median H2T

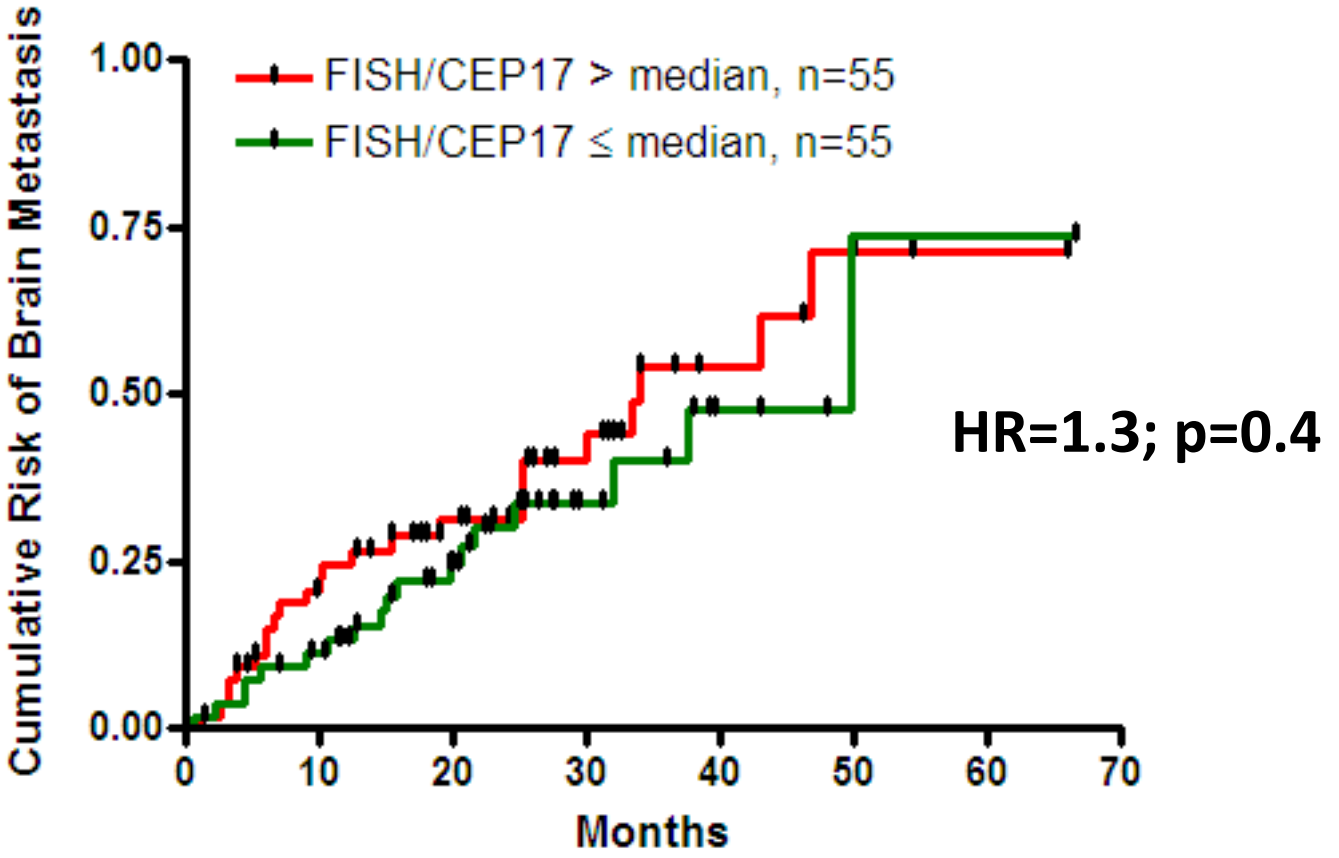


Time to brain metastases according to H2T cutoff of 50 RF/mm^2



HR = 2.6; p = 0.003

Time to brain metastases according to median HER2 FISH/CEP17



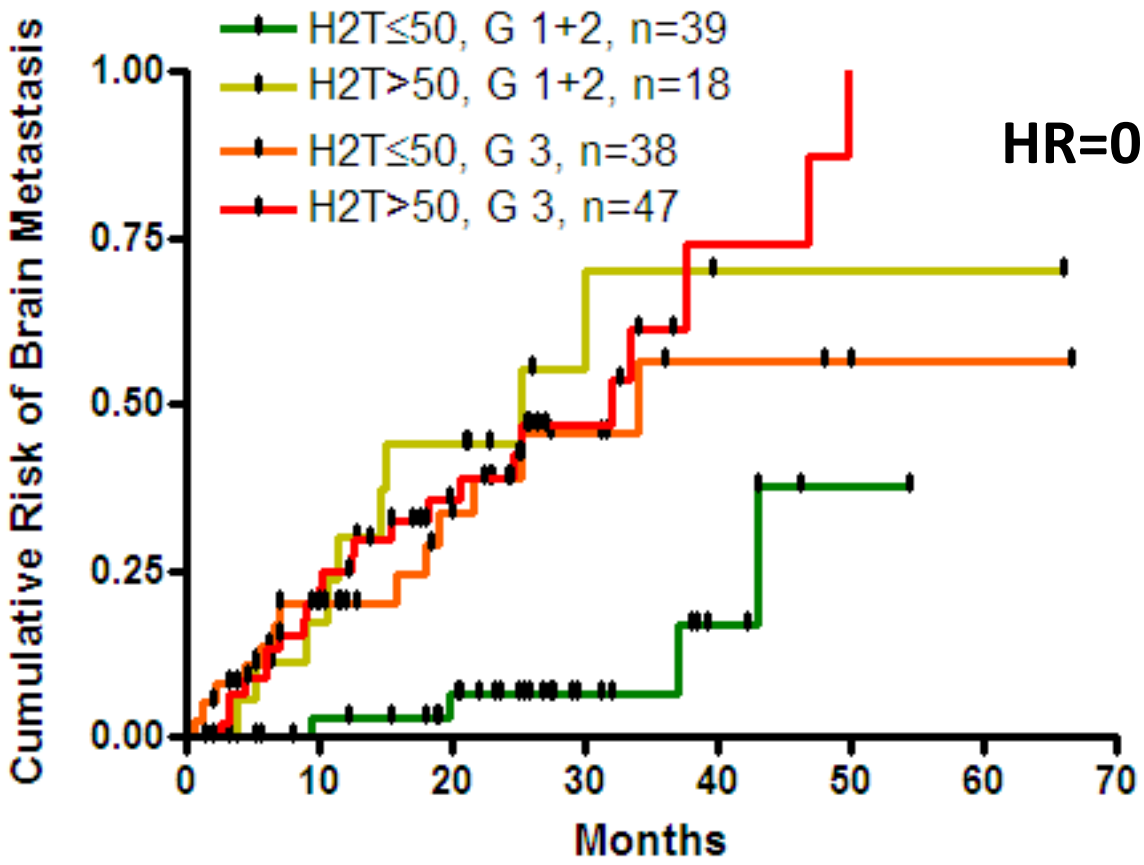
Time to brain metastases: univariate analysis stratified by grade

Variable	Category	HR	P
Age	Continuous	0.99	0.7
Menopausal status	Pre/Postmenopausal	1.06	0.8
Dominant metastases	Viscera/Bone/Soft tissue	-	0.088
ER	Positive/Negative	0.86	0.6
PgR	Positive/Negative	1.3	0.4
Grade	G3/G1 + G2	-	-
FISH HER2	Positive/Negative	1.4	0.6
Log FISH HER2	Continuous	1.4	0.5
Log HER2 protein (H2T)	Continuous	1.9	0.07
HER2 protein (H2T)	> 50/≤ 50	2.2	0.013
Time to non-brain metastases	Continuous	2.4	0.01

Time to brain metastases in HER2 FISH-positive patients: univariate analysis stratified by grade

Variable	Category	HR	P
Age	Continuous	1.0	1.0
Menopausal status	Pre/Postmenopausal	1.07	0.8
Dominant metastases	Viscera/Bone/Soft tissue	-	0.12
ER	Positive/Negative	1.1	0.9
PgR	Positive/Negative	1.5	0.22
Grade	G3/G1 + G2	-	-
Log FISH HER2	Continuous	1.2	0.7
Log HER2 protein (H2T)	Continuous	2.8	0.022
HER2 protein (H2T)	> 50/≤ 50	2.3	0.014
Time to non-brain metastases	Continuous	2.2	0.025

Time to brain metastases: low H2T and grade 1+2 vs. three other subsets



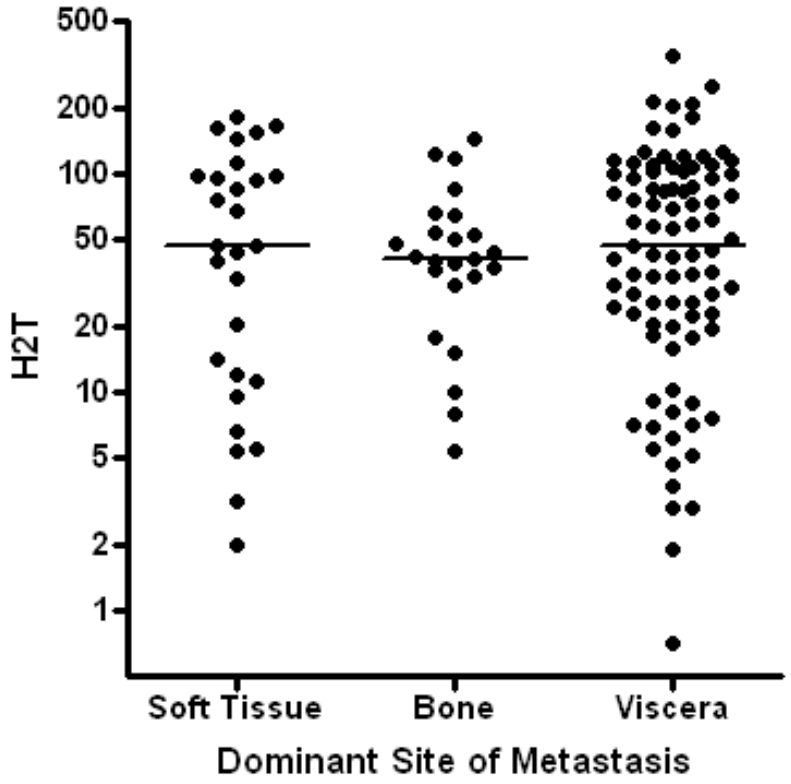
HR=0.17; p=0.0001

Multivariate analysis of time to brain metastases

Variable	All patients		FISH-positive	
	HR	p	HR	p
Log HER2 protein (H2T)	2.3	0.071	3.3	0.024
Log FISH HER2	0.61	0.46	0.45	0.32
Time to non-brain progression[a]	3.0	0.0035	2.9	0.0056

[a]Time to non-brain progression used as a time-dependent variable to examine effect of other progressions on risk of brain metastases

HER2 protein level (H2T) for each of three dominant metastatic sites



p=0.9

Also no correlation was found between the dominant metastatic site and time to brain metastases (p = 0.1)

Summary

- **The first study to demonstrate association between quantitatively expressed HER2 protein level and the risk for developing brain metastases in HER2-positive breast cancer patients**
- **HER2 protein level, tumor grade and time to any distant progression were the only independent predictors of brain relapse**
- **No impact of conventional HER2 FISH on brain relapse**
- **No correlation between HER2 protein level and the risk for developing extracranial metastases**

Interpretation

- **Effect of specific biology of HER2-overexpressing tumors?**
- **Reasons for FISH inefficacy to predict brain relapse:**
  - **Quantitative measure of functional protein (25,000 to 2,000,000 receptors/cell) with HERmark assay, vs. numeric count of *HER2* gene amplification (DNA) with FISH**
  - **DNA amplicon detected by the HER2 FISH probe contains a number of other biologically significant genes**
  - **Problems with FISH measurement in some cases (clusters)**
- **Differential biological effect of H2T in brain and extracranial sites may be due to inability of trastuzumab to penetrate the central nervous system**

Conclusions

- **HER2 protein expression in the primary tumor may identify HER2 positive trastuzumab-treated advanced breast cancer patients at particularly high risk of developing brain metastases**
- **These patients might potentially benefit from more personalized preventive and therapeutic strategies**
- **Study limitations:**
  - **retrospective design**
  - **limited sample size**
  - **lack of a trastuzumab-untreated control arm**
  - **lack of central IHC measurements to initially exclude HER2 false-positive cases**
- **These data warrant independent validation**

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Breast Cancer

Correlation Between Quantitative HER-2 Protein Expression and Risk for Brain Metastases in HER-2^{+} Advanced Breast Cancer Patients Receiving Trastuzumab-Containing Therapy

RENATA DUCHNOWSKA,[a,m] WOJCIECH BIERNAT,[b,m] BARBARA SZOSTAKIEWICZ,[b,m] JEFF SPERINDE,[c] FANNY PIETTE,[d] MOJGAN HADDAD,[c] AGNES PAQUET,[c] YOLANDA LIE,[c] BOGUMIŁA CZARTORYSKA-ARŁUKOWICZ,[e,m] PIOTR WYSOCKI,[f,m] TOMASZ JANKOWSKI,[g] BARBARA RADECKA,[h] MAŁGORZATA FOSZCZYŃSKA-KŁODA,[i,m] MARIA LITWINIUK,[j,m] SYLWIA DĘBSKA,[k,m] JODI WEIDLER,[c] WEIDONG HUANG,[c] MARC BUYSE,[d] MICHAEL BATES,[l] JACEK JASSEM[b,m]

[a]Military Institute of Medicine, Warsaw, Poland; [b]Medical University of Gdańsk, Gdańsk, Poland; [c]Monogram Biosciences, South San Francisco, California, USA; [d]International Drug Development Institute, Louvain-la-Neuve, Belgium; [e]Białystok Oncology Center, Białystok, Poland; [f]Great Poland Cancer Center, Poznań, Poland; [g]Lublin Oncology Center, Lublin, Poland; [h]Opole Oncology Center, Opole, Poland; [i]West Pomeranian Oncology Center, Szczecin, Poland; [j]Poznań University of Medical Sciences, Poznań, Poland; [k]Regional Cancer Center, Łódź, Poland, [l]Oncology Research and Development, Cepheid, Sunnyvale, California, USA; [m]Polish Brain Metastasis Consortium

The Oncologist 2012;17:26–35 www.TheOncologist.com